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HPLC Determination of Tolperisone in Human Plasma

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A simple high performance liquid chromatographic (HPLC) method was developed for the determination of tolperisone in human plasma. Tolperisone and internal standard (chlorphenesin) were isolated from 1 mL of plasma using 8 mL of dichlormethane. The organic phase was collected and evaporated under nitrogen gas. The residue was then reconstituted with 300 mL aliquot of mobile phase and a 100 mL aliquot was injected onto the C_{18} reverse-phased column. The mobile phase, 45% methanol containing 1% glacial acetic acid and 0.05% 1-hexanesulfonic acid was run at a flow rate of 1 mL/min. The column effluent was monitored using UV detector at 260 nm. The retention times for tolperisone and the internal standard were approximately 7.1 and 8.4 min, respectively. The standard curve was linear with minimal intra-day and inter-day variability. The quantification limit of tolperisone in human plasma was 10 ng/mL. The proposed method has been applied to the determination of pharmacokinetic profile of tolperisone in Koreans. The T_{max} of tolperisone in Koreans (0.94±0.42 h) was not significantly differ from that reported in Europeans (0.5-1 h), but the mean half-life in Koreans (1.14±0.27 h) was shorter than that in Europeans (2.56±0.2 h). The proposed HPLC method is simple, accurate, reproducible and suitable for pharmacokinetic study of tolperisone.

Key words: Tolperisone, Plasma, HPLC, Pharmacokinetics

INTRODUCTION

Tolperisone hydrochloride (dimethyl-2,4'-piperidino-3-propiophenone) is a centrally acting muscle relaxant (Fels, 1996; Zsila *et al.*, 2000) used for relieving spasticities of neurological origin and muscle spasms associated with painful locomotor diseases (Bajaj *et al.*, 2003; Svensson *et al.*, 2003). Although the precise mechanism is still unknown, it is believed that its action as a centrally acting muscle relaxant is principally based on the inhibition of the voltage gated sodium channels in the brain stem (Kocsis *et al.*, 2002).

The maintenance of effective drug concentration is very important to get the successful therapeutic result, and thus there has been an increasing demand for a suitable method for the assay of therapeutic drugs in biological samples. There are very few reports on assay methods of tolperisone, such as potentiometry (The Pharmacopoeia of Japan, 1986), spectrophotometry (The Pharmacopoeia of Japan, 1991), high performance thin layer chromatography (Liawruangrath and Liawruangrath, 1999), gas-liquid chromatography (Miskolczi *et al.*, 1987) and high performance liquid chromatography (HPLC) (Liawruangrath *et al.*, 2001). HPLC method using UV detector is most available in many laboratories. However, there is no report on HPLC determination of tolperisone in biological samples, because the previously reported HPLC method (Liawruangrath *et al.*, 2001) was for pharmaceuticals samples.

The aim of this work was to develop a new simple and sensitive HPLC method for the determination of tolperisone in human plasma. For the application of this method, the pharmacokinetic profile of oral tolperisone HCl in Koreans was also reported.

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MATERIALS AND METHODS

Materials

Tolperisone HCl (Mydocalm) was purchased from Hanlim

Pharm. Co. (Seoul, Korea). Methanol (HPLC grade) was obtained from J.T. Baker (Mallinckrodt Baker, Inc., Phillipsburg, NJ, U.S.A.). Chlorphenesin carbamate (internal standard, I.S.) and 1-hexanesulfonic acid were purchased from Sigma-Aldrich (St. Louis, Mo, U.S.A.).

Preparation of calibration standards and quality control samples

Primary stock solutions of tolperisone and I.S. were prepared in deionized water. Working solutions were prepared by diluting each stock solutions with deionized water. Calibration standards and Quality control (QC) samples of concentration range from 10 ng/mL to 750 ng/mL were prepared by spiking 100 μL of the appropriate working solution into 0.9 mL of drug-free human plasma.

Sample preparation

One hundred microliter of 100 mg/mL chlorphenesin carbamate (as I.S.) was added to a 1 mL of aliquot of blank plasma or calibration standards, and 8 mL of dichloromethane was added. The mixture was mixed for 30 s and centrifuged at 3,500 rpm for 10 min. The organic layer was transferred to a 10 mL tube and evaporated to dryness under nitrogen stream in a 50°C water bath. The residue was redissolved in 300 mL of mobile phase and a 100 μL aliquot was injected into the HPLC system.

HPLC instrumentation and conditions

The HPLC system consisted of a Waters 515 HPLC pump, a Waters 717 plus autosampler and a Waters 2487 Dual λ absorbance detector (Waters Assoc. Milford, MA, U.S.A.). The separation was performed on a Capcell Pak C18 reversed-phase column (5 μm, 4.6 i.d. × 150 mm, Shiseido, Tokyo, Japan) using a 45% methanol containing 1% glacial acetic acid and 0.05% 1-hexanesulfonic acid at a flow rate of 1 mL/min. The column temperature was 30°C and the detection wavelength was 260 nm.

Assay validation

The specificity of the method was determined by comparing the chromatograms obtained from the samples containing tolperisone and I.S. with those obtained from blank samples. Limit of quantification (LOQ) was defined as the lowest concentration at which the precision expressed by relative standard deviation is better than 20% and accuracy expressed by relative difference of the measured and true value is also lower than 20%. Precision was determined as the coefficient of variation (CV), and the accuracy as the percentage relative error (RE). Precision and accuracy data were obtained by analyzing aliquots of six spiked plasma at 10, 50, 100, 250, 500 and 750 ng/mL of tolperisone. Intra-day reproducibility was determined by analyzing 5 aliquots of spiked human plasma and inter-day reproducibility was determined over a 5-day period (n=5). The extraction recoveries of tolperisone were determined by comparing the peak area of three extracted samples at the concentration of 50 and 750 ng/mL with the mean peak area of recovery standards. To evaluate the three freeze/thaw cycle stability and room temperature matrix stability, six replicates of QC samples at each concentration of 50 and 750 ng/mL were subjected to three freeze/thaw cycles or were stored at room temperature for 12 h before processing, respectively. Six replicates of QC samples at each concentration of 50 and 750 ng/mL were processed and stored under autosampler conditions for 24 h were assayed to assess post-preparative stability.

Pharmacokinetic study

Eight healthy male Korean volunteers, weighing 66.7 ± 8.2 kg and aged 22.0 ± 1.5 years, were enrolled in a pharmacokinetic study of oral tolperisone HCl. This study was approved by the Institutional Review Board, and written informed consent was obtained from each subject. The subjects were not on any concomitant medications and were free from significant cardiac, hepatic, renal, pulmonary, gastrointestinal, neurological or hematological diseases, which was determined within four weeks prior to the beginning of the study by way of their medical history, physical examinations and laboratory screenings. They received a single oral dose of three 150 mg tolperisone HCl tablets at 8 a.m. after an overnight fast. No food was allowed for the following 4 hrs. Blood samples were collected in heparinized glass tubes from an antecubital vein immediately before administering tolperisone HCl (time zero) and at 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, and 5 h after administering the oral tolperisone HCl. The blood samples were immediately centrifuged, and the plasma sample was stored at -70°C until analysis.

Statistical analysis

The plasma drug concentration-time data from the study were analyzed using the BA-Calc 2002 program by Korea Food & Drug Administration. The area under the plasma concentration-time curve (AUC) and the half-life on the terminal phase ($t_{1/2}$) were calculated from the plasma drug concentration-time curve. The AUC_{0-t} was calculated with the trapezoidal method. The maximum plasma concentration (C_{max}) and the time required to reach the maximal plasma concentration (T_{max}) were recorded directly from the measured data.

RESULTS

HPLC conditions

Representative chromatograms of blank plasma sample,

plasma spiked with tolperisone HCl and plasma sample at 1.5 h after the oral administration of tolperisone HCl at a dose of 450 mg are presented in Fig. 1. Retention times of tolperisone and I.S. were approximately 7.1 and 8.4 min, respectively. There was no interfering peak from endogenous substances in the blank plasma. Retention time of tolperisone can be adjusted by changing the concentration of 1-hexanesulfonic acid in mobile phase (Fig. 2). 1-Hexanesulfonic acid was mixed at a concentration of 0.05% in mobile phase in this study.

Assay validation

The calibration curves were linear in the studied range. Calibration standards containing 10-750 ng/mL were prepared from working solutions of tolperisone and blank plasma. The calibration curve equation is y=bx+c, where y represents the tolperisone peak area to I.S. peak area ratio and x represents the ratio of tolperisone concentration. The mean equation of the calibration curve (N=10) obtained from 6 points was y = 0.0031x + 0.0029 (correlation coefficient r^2=1). The limit of quantification was 10 ng/mL.

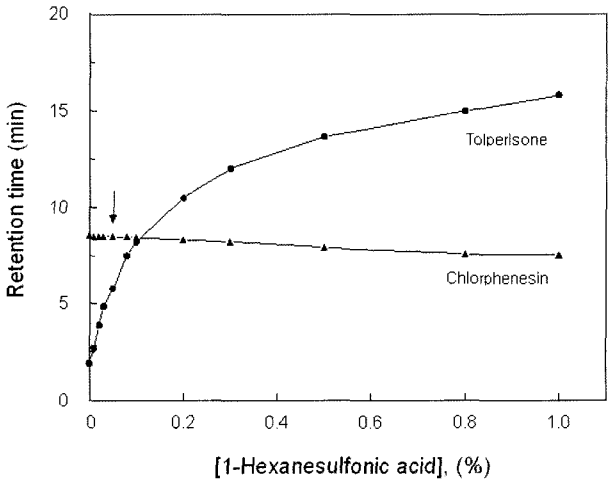

Fig. 2. Effect of 1-hexanesulfonic acid on the retention time of tolperisone and chlorphenesin

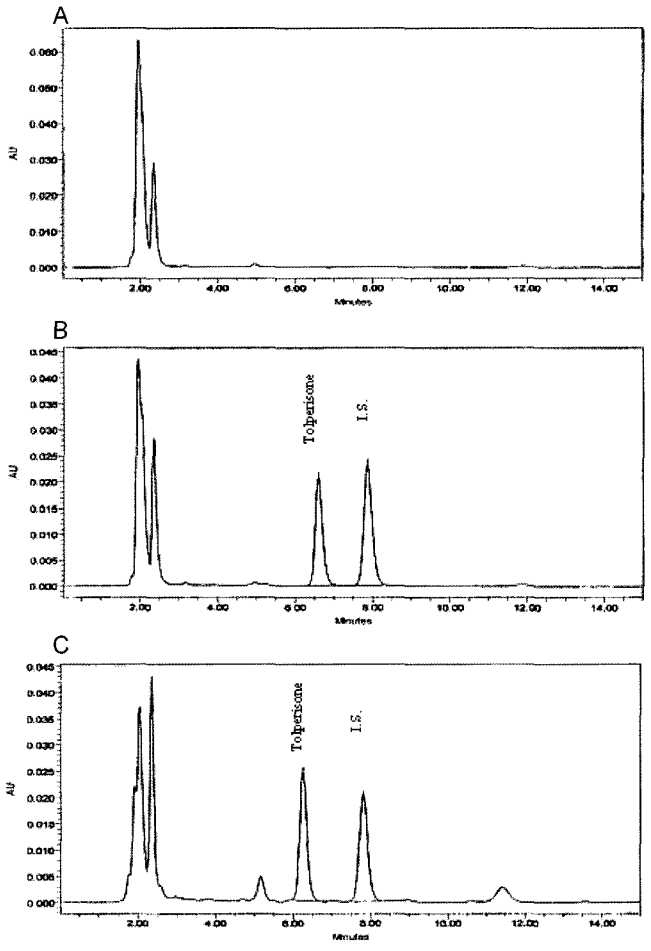

Fig. 1. HPLC chromatogram of plasma tolperisone and internal standard (I.S., chlorphenesin carbamate). A: blank human plasma, B: human plasma spiked with tolperisone (250 ng/mL) and I.S. (100 ng/mL), C: human plasma sample at 1.5 h after administration of tolperisone HCl 450 mg

The intra- and inter-day precision and accuracy of the developed method were evaluated with nine replicates of samples at concentration of 10 to 750 ng/mL, and on five different days (Table I). The ranges of intra- and inter-day coefficients of variations (CVs) were 0.4-3.43 and 0.58-4.22%, respectively. The intra-day accuracy were 91-100%.

The extraction recoveries of tolperisone at 50 and 750 ng/mL were 96.8 ± 2.3 and 97.1 ± 2.4%, respectively. The stability of tolperisone during sampling handling and the stability of processed samples were evaluated. Three freeze-thaw cycles and room temperature storage of the QC samples for 12 h before analysis did not significantly affect the quantification (Data were not shown). Extracted QC samples were allowed to stand at room temperature for 24 h prior to injection without affecting the quantification (Data were not shown).

Table I. Precision and accuracy of HPLC assay method for plasma tolperisone (n=5)

Concentration (ng/mL)	Precision (CV%)		Accuracy (%)
	Intra-day	Inter-day	
10	3.43	4.22	91
50	1.02	1.57	96
100	0.40	0.93	99
250	0.92	0.58	100
500	0.87	1.17	99
750	0.80	1.46	98

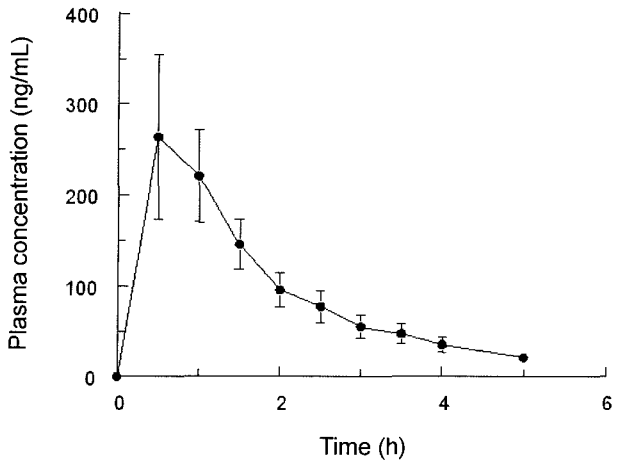

Fig. 3. Plasma concentration of tolperisone after oral administration. Each value represents the Mean ± S.D. of 8 volunteers.

Table II. Pharmacokinetic parameters of oral tolperisone HCl in 8 volunteers

AUC (ng/mL h)	C_{max} (ng/mL)	T_{max} (h)	Ke (h^{-1})	$T_{1/2}$ (h)
516.0±335.7	297.6±240.4	0.94±0.42	0.620±0.198	1.14±0.27

Each value represents the mean ± S.D..

Pharmacokinetic study

The proposed method was applied to the determination of tolperisone in plasma samples for purpose of the pharmacokinetic study. Plasma samples were periodically collected up to 5 h after administration of tolperisone HCl at a single oral dose of 450 mg to 8 healthy male volunteers. Plasma concentration-time curve of tolperisone after oral administration tolperisone is shown in Fig. 3. The pharmacokinetic parameters and statistical moment parameters of tolperisone is summarized in Table II. Absorption and distribution after oral administration of tolperisone HCl appeared to be rapid. Peak plasma concentrations of 297.6 ± 240.4 ng/mL were abtained within 0.94 ± 0.42 h. The mean half-life was 1.14 ± 0.27 h and $AUC_{0\text{-}5h}$ 516.0 ± 335.7 ng/mL·h.

DISCUSSION AND CONCLUSIONS

This is the first report for HPLC determination of tolperisone in human plasma samples. The method described in this report gave a very clean chromatogram in which tolperisone and internal standard peaks were well enough resolved. In addition, peak of tolperisone can be adjusted by changing the concentration of 1-hexanesulfonic acid in mobile phase. The detection limit of this method for tolperisone is 10 ng/mL. This method demonstrated the acceptable precision, accuracy, recovery and stability, and could be very convenient and useful for bioequivalence test and pharmacokinetic studies for tolperisone.

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